



SEQUENCE LISTING

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<110> THASTRUP, Ole  
TULLIN, Soren  
POULSEN, Lars K  
BJORN, Sara P

<120> Novel Florescent Proteins

<130> 3759-0106P

<140> 09/619,310

<141> 2000-07-19

<150> US 08/819,612

<151> 1997-03-17

<150> PCT/DK96/00051

<151> 1996-01-31

<150> DK 1065/95

<151> 1995-09-22

<160> 23

<170> PatentIn version 3.1

<210> 1

<211> 36

<212> DNA

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<223> GFP2 primer directed to A. victoria

<400> 1

tggaataagc tttatgagta aaggagaaga actttt

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36

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<223> 5' PCR primer incorporating the Y66H substitution responsible for changing green fluorescence into blue fluorescence

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gataccc 67

<210> 4  
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<223> 3' PCR primer incorporating the Y66H substitution responsible for changing green fluorescence into blue fluorescence

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<212> DNA  
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<220>  
<223> 5' primer used to flank the Y66H-GFP

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ctttcgtttt gaattcggat cccttttagtg 30

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<223> large NcoI-XbaI vector fragment and ligated to SEQ ID NO:8

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<210> 8  
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Leu Val Glu Leu Asp Gly Asp Val Asn Gly Gln Lys Phe Ser Val Ser  
15 20 25 30

gga gag ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa ttt 145  
Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe  
35 40 45

att tgc act act ggg aag cta cct gtt cca tgg cca acg ctt gtc act 193  
Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr  
50 55 60

act ttc tct tat ggt gtt caa tgc ttt tca aga tac cca gat cat atg 241

Thr	Phe	Ser	Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met	
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Lys	Gln	His	Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	
	80					85					90					
gaa	aga	act	ata	ttt	tac	aaa	gat	gac	ggg	aac	tac	aag	aca	cgt	gct	337
Glu	Arg	Thr	Ile	Phe	Tyr	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	
95					100					105					110	
gaa	gtc	aag	ttt	gaa	ggg	gat	acc	ctt	gtt	aat	aga	atc	gag	tta	aaa	385
Glu	Val	Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	
				115					120					125		
ggg	att	gat	ttt	aaa	gaa	gat	gga	aac	att	ctt	gga	cac	aaa	atg	gaa	433
Gly	Ile	Asp	Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Met	Glu	
			130					135					140			
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Tyr	Asn	Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile	Met	Ala	Asp	Lys	Pro	Lys	
		145					150					155				
aat	gga	atc	aaa	gtt	aac	ttc	aaa	att	aga	cac	aac	att	aaa	gat	gga	529
Asn	Gly	Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn	Ile	Lys	Asp	Gly	
	160					165					170					
agc	gtt	caa	tta	gca	gac	cat	tat	caa	caa	aat	act	cca	att	ggc	gat	577
Ser	Val	Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	Pro	Ile	Gly	Asp	
	175				180					185					190	
ggc	cct	gtc	ctt	tta	cca	gac	aac	cat	tac	ctg	tcc	acg	caa	tct	gcc	625
Gly	Pro	Val	Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu	Ser	Thr	Gln	Ser	Ala	
				195					200					205		
ctt	tcc	aaa	gat	ccc	aac	gaa	aag	aga	gat	cac	atg	atc	ctt	ctt	gag	673
Leu	Ser	Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp	His	Met	Ile	Leu	Leu	Glu	
			210					215					220			
ttt	gta	aca	gct	gct	ggg	att	aca	cat	ggc	atg	gat	gaa	cta	tac	aaa	721
Phe	Val	Thr	Ala	Ala	Gly	Ile	Thr	His	Gly	Met	Asp	Glu	Leu	Tyr	Lys	
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taa	atg	tcca	gact	tcca	at	tgac	actaaa	ggg	atc	cgaa	ttc					764

<210> 16  
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 <212> PRT  
 <213> Aequeorea Victoria

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20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys  
35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe  
50 55 60


Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln  
65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg  
85 90 95

Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val  
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile  
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu Tyr Asn  
130 135 140

 Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys Asn Gly  
145 150 155 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp Gly Ser Val  
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro  
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser  
195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu Phe Val  
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Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys  
225 230 235

<210> 17

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ctt gtt gaa tta gat ggc gat gtt aat ggg caa aaa ttc tcc gtt agt 97  
 Leu Val Glu Leu Asp Gly Asp Val Asn Gly Gln Lys Phe Ser Val Ser  
 15                  20                  25                  30

gga gag ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa ttt 145  
 Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe  
                           35                  40                  45

att tgc act act ggg aag cta cct gtt cca tgg cca acg ctt gtc act 193  
 Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr  
                   50                  55                  60

act ctc tct cat ggt gtt caa tgc ttt tct aga tac cca gat cat atg 241  
 Thr Leu Ser His Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met  
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gaa aga act ata ttt tac aaa gat gac ggg aac tac aag aca cgt gct 337  
 Glu Arg Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala  
           95                  100                  105                  110

gaa gtc aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa 385  
 Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys  
                   115                  120                  125

ggt att gat ttt aaa gaa gat gga aac att ctt gga cac aaa atg gaa 433  
 Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu  
           130                  135                  140

tac aat tat aac tca cat aat gta tac atc atg gca gac aaa cca aag 481  
 Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys  
           145                  150                  155

aat ggc atc aaa gtt aac ttc aaa att aga cac aac att aaa gat gga 529  
 Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp Gly  
           160                  165                  170

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 Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp

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Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala				
	195	200	205	
ctt tcc aaa gat ccc aac gaa aag aga gat cac atg atc ctt ctt gag				673
Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu				
	210	215	220	
ttt gta aca gct gct ggg att aca cat ggc atg gat gaa cta tac aaa				721
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	225	230	235	
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<400> 18

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
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Glu Leu Asp Gly Asp Val Asn Gly Gln Lys Phe Ser Val Ser Gly Glu
20 25 30

D3

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
50 55 60

Ser His Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
85 90 95

Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu Tyr Asn



130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys Asn Gly  
 145 150 155 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp Gly Ser Val  
 165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro  
 180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser  
 195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu Phe Val  
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Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys  
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 Leu Val Glu Leu Asp Gly Asp Val Asn Gly Gln Lys Phe Ser Val Ser  
 15 20 25 30

gga gag ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa ttt 145  
 Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe  
 35 40 45

att tgc act act ggg aag cta cct gtt cca tgg cca acg ctt gtc act 193  
 Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr  
 50 55 60

act ctc tct tat ggt gtt caa tgc ttt tct aga tac cca gat cat atg 241  
 Thr Leu Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met

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gaa gtc aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys 115 120 125			385
ggt att gat ttt aaa gaa gat gga aac att ctt gga cac aaa atg gaa Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu 130 135 140			433
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aat ggc atg gaa tac aat tat aac tca cat aat gta tac atc atg gca Asn Gly Met Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala 160 165 170			529
gac aaa cca aag aat ggc atc aaa gtt aac ttc aaa att aga cac aac Asp Lys Pro Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn 175 180 185 190			577
att aaa gat gga agc gtt caa tta gca gac cat tat caa caa aat act Ile Lys Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr 195 200 205			625
cca att ggc gat ggc cct gtc ctt tta cca gac aac cat tac ctg tcc Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser 210 215 220			673
acg caa tct gcc ctt tcc aaa gat ccc aac gaa aag aga gat cac atg Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met 225 230 235			721
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35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu  
50 55 60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln  
65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg  
85 90 95

Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val  
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile  
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu Tyr Asn  
130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys Asn Gly  
145 150 155 160

Met Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys  
165 170 175

Pro Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys  
180 185 190

Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile  
195 200 205

Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln  
210 215 220

Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu  
 225 230 235 240

Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu  
 245 250 255

Tyr Lys

<210> 21  
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                   35                  40                  45  
 att tgc act act ggg aag cta cct gtt cca tgg cca acg ctt gtc act 193  
 Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr  
                   50                  55                  60  
 act ctc act tat ggt gtt caa tgc ttt tct aga tac cca gat cat atg 241  
 Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met  
                   65                  70                  75  
 aaa cag cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta cag 289  
 Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln  
                   80                  85                  90  
 gaa aga act ata ttt tac aaa gat gac ggg aac tac aag aca cgt gct 337  
 Glu Arg Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala  
                   95                  100                  105                  110  
 gaa gtc aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa 385  
 Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys  
                   115                  120                  125  
 ggt att gat ttt aaa gaa gat gga aac att ctt gga cac aaa atg gaa 433

Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu	
130 135 140	
tac aat tat aac tca cat aat gta tac atc atg gca gac aaa cca aag	481
Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys	
145 150 155	
aat ggc atc aaa gtt aac ttc aaa att aga cac aac att aaa gat gga	529
Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp Gly	
160 165 170	
agc gtt caa tta gca gac cat tat caa caa aat act cca att ggc gat	577
Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp	
175 180 185 190	
ggc cct gtc ctt tta cca gac aac cat tac ctg tcc acg caa tct gcc	625
Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala	
195 200 205	
ctt tcc aaa gat ccc aac gaa aag aga gat cac atg atc ctt ctt gag	673
Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu	
210 215 220	
ttt gta aca gct gct ggg att aca cat ggc atg gat gaa cta tac aaa	721
Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys	
225 230 235	
taa atgtccagac ttccaattga cactaaaggg atccgaattc	764

<210> 22  
 <211> 238  
 <212> PRT  
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<400> 22

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35 40 45	
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu	
50 55 60	
Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln	
65 70 75 80	

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg  
85 90 95

Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val  
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile  
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu Tyr Asn  
130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys Asn Gly  
145 150 155 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp Gly Ser Val  
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro  
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser  
195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu Phe Val  
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Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys  
225 230 235

<210> 23  
<211> 14  
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